In Vitro Determination of Chlorophyll a and Pheophytin a in Marine and Freshwater Algae by Fluorescence EPA 445.0

Facility Name:_____VELAP ID_____

Assessor Name:_____ Analyst Name:_____ Inspection Date_____

Relevant Aspect of Standards	Method Reference	Y	N	N/A	Comments
Records Examined: SOP Number/ Revision/ Date _____ Analyst:_____ Sample ID: _____ Date of Sample Preparation:_____ Date of Analysis:_____					
Has the lab established an MDL for chlorophyll a?	1.2				
Has the lab established an LDR for chlorophyll a using a minimum of 5 calibration standards ranging in concentration from 0.2ug/L to 200 ug a/L across all sensitivity settings of the fluorometer?	9.2.2				
Is dilution used instead of minimum sensitivity settings on the fluorometer when quenching occurs due to highly concentrated solutions?	4.3				
Are samples, standards, LRBs and QCSs analyzed at the same temperature (ambient is recommended with fluctuations of less than ±3°C) ?	4.4				
Are samples clarified by centrifugation prior to analysis?	4.5				
Are Standards, QC materials and filter samples stored in the dark at -20°C or -70±°C and is work performed in subdued light to prevent degradation?	4.5				
Is HPLC grade acetone and ASTM Type I water used?	7.1 & 7.4				
Is the concentration of the Chlorophyll Stock Standard Solution (SSS) determined spectrophotometrically, stored in a light and airtight container in the freezer? Is the concentration of all SSS dilutions determined spectrophotometrically each time they are made?	7.7				
Is the LRB the last filter extracted of the sample set?	7.8				
Is the Chlorophyll a Primary Dilution Standard (PDS) prepared fresh just prior to use?	7.9				
Is sample filtering performed in subdued light at a vacuum of less than 6in. Hg (20KPa) as soon as possible, preferably aboard ship?	8.1				
Prior to filtration, is the sample container thoroughly but gently agitated to suspend particulates? And is the vacuum slowly released on the filter as the last bit of water is pulled through?	8.1				
Is the filter placed in a container wrapped in aluminum foil and stored no more than 2-4hr on ice, then at -20°C or -70±°C in the dark until extraction (no more than 3.5 weeks)?	8.1 - 8.3				
Is the Estimated Detection Limit (EDL) determined by analyzing a pure chlorophyll a solution in 90% acetone which has been serially diluted until it yields a response which is 3X the average response of several blank filters?	9.2.4				
Is the extraction proficiency for each new analyst determined using 20-30	9.2.6				

samples and evaluating the percent relative standard deviation (%RSD) of uncorrected values of chlorophyll a (not to exceed 15% for samples that are 10X the IDL)?					
Is the ability for new analysts to adequately mix the acidified sample evaluated using 5-10 separate aliquots of a 50ppb chl a solution in 90% acetone, using separate cuvettes yielding a <5%RSD for the corrected chl a?	9.2.7				
Is a (mandatory) filter blank extracted at the end of each sample set and evaluated to be less than 10% of the analyte level?	9.3				
Is the calibration performed bimonthly or when there has been an adjustment made to the instrument, using 5 standards (0.2-200 ug chl a/L) prepared from the PDS?	10.0				
Are samples placed in glass grinding tubes, to which 4 mL of aqueous acetone is added and ground to a slurry (without overheating)?	11.1.1				
Is the slurry then transferred to a 15mL screw cap centrifuge tube and the pestle and grinding tube rinsed with 6mL of 90% acetone (added to slurry)?	11.1.1				
Is the centrifuge tube vigorously shaken and placed in the dark until all samples are extracted?	11.1.1				
Is the pestle, grinding tube and glass rod rinsed with acetone and water (always acetone last) and wiped between sample extractions ?	11.1.1				
Are samples shaken again vigorously before steeped in the dark at 4C for 2-24hours (and shaken at least once during steeping period)?	11.1.2				
After steeping, is each sample tube shaken vigorously and centrifuged for 15 minutes at 675g or for 5 minutes at 1000g and allowed to come to room temperature?	11.1.3				
Is the fluorometer allowed to warm for at least 15 min and zeroed using the 90% acetone solution at the sensitivity that will be used for sample analysis?	11.2.1				
Are sample supernatants poured/pipette into cuvettes and read at a sensitivity setting that yields a midscale reading when possible (and diluted when the concentration of the chlorophyll a is >90% of the upper limit of the LDR)?	11.2.2				
FOR PHEOPHYTIN					
After the fluorescence measurement and sensitivity setting used for the sample is recorded for chlorophyll a, is the cuvette removed from the fluorometer and acidified to a final concentration of 0.003 N HCl using 0.1 N HCl solution?	11.2.2				
After the acid is added and mixed thoroughly in the cuvette, is the sample held for 90 seconds before again reading on the fluorometer?	11.2.2				
CALCULATIONS					
Is the corrected, un-corrected chlorophyll a and pheophytin calculated according to 12.1, 12.2 and 12.3 respectively?	12.1-3				
Notes/ Comments:					